



# MAb Anti-6HIS-Tb cryptate

## PROTOCOL

**Part # 61HISTLF, 61HISTLA & 61HISTLB**

**Test size: 1,000 tests (61HISTLF) - 5,000 tests (61HISTLA) - 20,000 tests (61HISTLB)**

**Assay volume: 20 µL**

**Revision: 04 (May 2018)**

**Store at 2-8°C**

**For research use only.**

**Not for use in therapeutic or diagnostic procedures.**

### REAGENT DESCRIPTION

In an HTRF protein:protein interaction assay, one protein is labeled (directly or indirectly) with the donor, and the other protein is labeled (directly or indirectly) with the acceptor. When the two proteins interact, the donor molecule is brought into proximity with the acceptor molecule. Excitation of the donor will result in signal generation proportional to the binding of proteins.

Mouse monoclonal antibody labeled with Lumi4® Tb is an IgG2a raised against a polyhistidine tagged fusion protein. It recognizes synthetic polyhistidine or polyhistidine-tagged fusion protein.

### MATERIALS

| Reagent                                                                                                                  | 1,000 tests | 5,000 tests | 20,000 tests |
|--------------------------------------------------------------------------------------------------------------------------|-------------|-------------|--------------|
| MAb Anti-6HIS-Tb cryptate<br>Lyophilized<br>In phosphate buffer pH 7.0 containing<br>protease free bovine serum albumin. | 1 vial      | 1 vial      | 1 vial       |

| Cisbio reagents Not provided                            | Part #           |
|---------------------------------------------------------|------------------|
| PPI - Terbium detection buffer<br>200 mL - ready-to-use | Cat # 61DB10RDF* |
| Plates* - HTRF 96-well low volume plate                 | 66PL96001        |

\*For HTRF microplate recommendations, please visit <http://www.cisbio.com/microplate-recommendations>

For reading, an HTRF®-compatible reader is needed. Make sure to use the appropriate setup.

For a list of HTRF®-compatible readers and setup recommendations, please visit <http://www.cisbio.com/readers>

## STORAGE AND STABILITY

- Store the reagent at 2-8°C.
- Under appropriate storage conditions, reagents are stable until the expiry date indicated on the label.
- Once reconstituted, stock solutions are stable for two days at 2-8°C. They can be refrozen (at  $\leq -16^{\circ}\text{C}$ ) and thawed once only.
- Do not repeat freezing and thawing.

## ASSAY FORMAT

When used as suggested, one vial from the two available sizes will provide sufficient reagent for 1,000 tests, 5,000 tests and 20,000 tests respectively, using a 20  $\mu\text{L}$  final assay volume. Assay volumes can be adjusted proportionally to run the assay in 96- or 1536-well microplates.

|                                     | Volume           |
|-------------------------------------|------------------|
| Other assay components              | 10 $\mu\text{L}$ |
| Acceptor (d2 or XL665) conjugate    | 5 $\mu\text{L}$  |
| Donor (Eu or Tb Cryptate) conjugate | 5 $\mu\text{L}$  |
| Final volume*                       | 20 $\mu\text{L}$ |

## REAGENT HANDLING

### BUFFERS

Cisbio PPI - Terbium detection buffer Cat # 61DB10RDF\* has been optimized for maximum performance.

It is mandatory to use the same buffer to prepare the donor and the acceptor (d2 or XL665) conjugates.

When using specific in-house buffers for the preparation of working solutions, we recommend a basic buffer such as PBS (Phosphate Buffered Saline) or Hepes with a pH maintained between 5.5 and 8.5.

It can be supplemented with BSA (0.1%), and detergents such as Tween 20, Triton X100 or CHAPS (up to 0.5%) to prevent reagent coating. Avoid SDS, due to its denaturing effect on XL665.

Please note that a phosphate-free buffer must be used in biochemical kinase assays to prevent interferences pertaining to the binding of anti-phospho specific antibodies.

Use of Tb cryptate donor does not require KF

### CONJUGATES

Allow each vial of lyophilized conjugate to warm up at room temperature.

Reconstitute the lyophilisate following the following instructions:

| MAB Anti-6HIS-Tb cryptate* | Stock solution preparation                   | Working solution preparation (see assay format above)                                                                                           |
|----------------------------|----------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| 1,000 tests                | Add 0.25 mL of distilled water<br>Mix gently | Dilute 20-fold the stock solution in PPI detection buffer<br>Mix gently<br>E.g. Add 4.75 mL PPI detection buffer to 0.25 mL of stock solution   |
| 5,000 tests                | Add 0.25 mL of distilled water<br>Mix gently | Dilute 100-fold the stock solution in PPI detection buffer<br>Mix gently<br>E.g. Add 24.75 mL PPI detection buffer to 0.25 mL of stock solution |
| 20,000 tests               | Add 1 mL of distilled water<br>Mix gently    | Dilute 100-fold the stock solution in PPI detection buffer<br>Mix gently<br>E.g. Add 99 mL PPI detection buffer to 1 mL of stock solution       |

\*Additional info is included in the batch information provided with the reagent.

The optimal amount per well will be dependent on assay conditions. For additional information on assay optimization, please refer to the technical note: [Guidelines for optimizing protein : protein interaction assays using HTRF PPI reagents.](#)

Make sure to prepare stock and working solutions according to the instructions that correspond to the packaging you have purchased (number of tests).

This product contains material of biological origin. Use for research purposes only. Do not use in humans or for diagnostic purposes. The purchaser assumes all risk and responsibility concerning reception, handling and storage.

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Europe and other countries +33(0)466-796-705 U.S. and Canada 1-888-963-4567 China +86-21-5018-9880  
Japan +81-(0)43-306-8712 Visit [www.cisbio.com](http://www.cisbio.com) to find a list of our regional distributors

